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Award Number: DAMD17-01-1-0037

TITLE: Human Prostate Cancer in a Tissue Recombination Model

PRINCIPAL INVESTIGATOR: Karin Williams, Ph.D.
Simon Hayward, Ph.D.

CONTRACTING ORGANIZATION: Vanderbilt University Medical Center
Nashville, Tennesse 37203-6917

REPORT DATE: December 2003

TYPE OF REPORT: Annual Summary

PREPARED FOR: U.S. Army Medical Research and Materiel Command
Fort Detrick, Maryland 21702-5012

REPORT DOCUMENTATION PAGE

Form Approved
OMB No. 074-0188

1. AGENCY USE ONLY (Leave blank)	2. REPORT DATE	3. REPORT TYPE AND DATES COVERED
	December 2003	Annual Summary (16 Apr 01-30 Nov 03)

4. TITLE AND SUBTITLE
Human Prostate Cancer in a Tissue Recombination Model

5. FUNDING NUMBERS
DAMD17-01-1-0037

6. AUTHOR(S)
Karin Williams, Ph.D.
Simon Hayward, Ph.D.

7. PERFORMING ORGANIZATION NAME(S) AND ADDRESS(ES)
Vanderbilt University Medical Center
Nashville, Tennesse 37203-6917

E-Mail: Karin.williams@vanderbilt.edu

8. PERFORMING ORGANIZATION REPORT NUMBER

9. SPONSORING / MONITORING AGENCY NAME(S) AND ADDRESS(ES)
U.S. Army Medical Research and Materiel Command
Fort Detrick, Maryland 21702-5012

10. SPONSORING / MONITORING AGENCY REPORT NUMBER

11. SUPPLEMENTARY NOTES
Original contains color plates. All DTIC reproductions will be in black and white.

12a. DISTRIBUTION / AVAILABILITY STATEMENT
Approved for Public Release; Distribution Unlimited

12b. DISTRIBUTION CODE

13. ABSTRACT (Maximum 200 Words)

In vivo models based on human epithelial cells (hPrE) provide powerful tools with which to investigate cancer initiation and progression. Tissue recombinations (TR) composed of hPrE and rat urogenital sinus mesenchyme (rUGM) grafted beneath the renal capsule of immunocompromised rodent hosts recapitulate many key events in prostatic development and adult function. The stable integration of known or putative oncogenes into the hPrE component of such TRs is a powerful tool with which to study the effects of these genes in vivo. The cMYC gene (a known oncogene) has been introduced into hPrE. cMYC is expressed in a very tightly regulated manner in non-malignant prostate but is deregulated in PIN and overexpressed/deregulated in high-grade human prostate cancer, suggesting a role in both the pathogenesis of PIN and subsequent progression to cancer.

The high efficiency LZRS/Phoenix retroviral system was used to insert the cMYC gene into hPrE under the control of the constitutively active CMV promoter. TR composed of infected hPrE and rUGM were made and grafted into SCID hosts. Hosts were sacrificed after carrying the grafts for 28 days.

We have visualized reporter and transgene expression. The TR's showed a rapidly growing metastatic PSA-expressing adenocarcinoma.

14. SUBJECT TERMS
Tissue Recombinant, Oncogene, Retrovirus, Tumor Biology, Prostatic Carcinogenesis

15. NUMBER OF PAGES
11

16. PRICE CODE

17. SECURITY CLASSIFICATION OF REPORT	18. SECURITY CLASSIFICATION OF THIS PAGE	19. SECURITY CLASSIFICATION OF ABSTRACT	20. LIMITATION OF ABSTRACT
Unclassified	Unclassified	Unclassified	Unlimited

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Statement of Work

The Investigation of Human Prostate cancer using a tissue recombination model

Task 1 To transfect human prostatic epithelium with cmv-ΔSV40, PB-ΔSV40, CMV-TSPY, PB-TSPY, PB-GFP and CMV-GFP and recombine with rUGM, followed by grafting into an athymic rat host. (Months 1-12)

a. Analysis of transfected cells in culture via EGFP (months 1-6)
b. Characterization of recombined transfected huPrE with rUGM and conformation of human prostatic cell architecture. (months 1-8)
c. Access the histological data and identify histologically altered cells (months 3-24)
d. Creation of cell lines from tissue removed from grafts at various time points and from any lesions observed (months 3-24)

Task 2 Steroid receptor localization and quantification in grafts consisting of untransfected huPrE, transfected huPrE both before and after castration (months 8-24)

a. Identification and analysis of ERß isoforms in the huPrE by IHC, western blotting and in situ hybridization (months 8-18)
b. Quantification of ERα immunoexpression and localization (months 12-24)
c. Analysis of AR expression, and AR mutation analysis by RT-PCR (months 12-24)
d. Investigation of PR expression in grafts both before and after castration (months 12-24)

Task 3 Analysis of apoptosis after hormonal depravation caused by castration (months 18-36)

a. Identification of hormone sensitive/insensitive cells within the recombinant grafts by use of the TUNEL assay (months 18-24).
b. Formation of cell lines derived from hormone insensitive cells (months 18-24)

Task 4 To identify genetic changes that associate with an altered phenotype within our huPrE/rUGS grafts (months 6-36)

a. Karyotyping of all cell lines derived from lesions observed in experimental animals and grafts (months 18-24)
b. CGH analysis of cell lines derived from metastatic tumors (months 6-18)
c. RT-PCR analysis combined with DNA sequencing of candidate oncogenes or tumor supressor genes (months 6-24)

Introduction

We are using a model in which **Human Prostatic Epithelial cells (huPrE)** are grown as a tissue recombinant with rat urogenital sinus mesenchyme (rUGM) and grafted back into the in vivo environment of an intact male athymic host.

The analysis of cells in culture carrying various constructs including TSPY and SV40T has been previously reported. To date grafts containing huPrE with TSPY have displayed no abnormal architecture possibly due to a long latency before a phenotype is observed in the human prostate and also as a result of low numbers of infected cells surviving within the graft. While some SV40T infected huPrE cells were obtained the cells were not immortal and failed to amplify sufficiently to allow TR's to be produced. Due to the nature of the SV40T and its non physiological contribution to human prostate cancer we decided to expand our studies to add an investigation of cMYC which is known to play a role in human prostate cancer but the mechanism by which it induces PIN and prostate cancer is still surprisingly unknown.

Key accomplishments

We have generated a new model of Prostate Cancer. This uses an oncogene (cMYC), naturally expressed in Prostate Cancer to drive transformation of human prostatic epithelial cells in vivo. The resulting tumors are rapidly growing, metastatic and express both PSA and androgen receptors.

- Tissue array analysis of human prostates are underway using arrays developed by Vanderbilt pathologists. Preliminary results for ERβ and the androgen regulated tumor suppressor Nkx3.1 (Fig.1)
- Retroviral constructs have been generated that allow transfer of cMYC into huPrE alongside the color marker EGFP (Fig. 2). Cells then self select to give pure populations thus removing the necessity to FACS the large fragile huPrE cells.
- The tissue culture conditions under which huPrE is infected with LZRS virus have been further optimized in order to increase the retroviral gene transfer potential of the system. In the case of cMYC the cells enter the cell cycle upon infection enhancing viral DNA integration allowing for 40-80% infection.
- Xenografts of huPrE cMYC and rUGM were grafted for 28 days, the host had to be sacrificed at this point due to tumor burden. The gross anatomy of the graft is shown in (Fig. 3). H+E of this graft shows many abnormalities in cell division.
- Xenografts of [huPrE +cMYC-Ires-EGFP] and rUGM at 1 month (Fig.4) have undergone IHC analysis. PSA expression within the cMYC containing grafts is visible within the adenocarcinoma confirming a prostatic origin for the adenocarcinoma.
- Immunocytochemistry and western blotting has confirmed the expression of the 'retrovirally introduced transgene' (Fig. 4 & 5) in both tissue culture and the resulting cMYC/EGFP expressing graft.
- Western blotting has likewise been utilized to identify expression of the cMYC protein (Fig. 5) and EGFP (Fig. 5) within the cMYC/EGFP graft.
- Western blot analysis on the tissue recombinant has detected Androgen Receptor (AR) expression as well as down regulation of the tumor suppressor PTEN, up regulation of the oncogene cMYB and down regulation of E-Cadherin (Fig.5).

- cDNA microarray analysis of both benign cells in culture paired with a pure population of cMYC overexpressing cells has been undertaken. We are at present confirming the true identity of the differentially regulated genes isolated in this screen

- The components of the 4th generation of LZRS constructs are approaching completion. This generation of constructs restricts expression of the transgene to the fully differentiated huPrE luminal cells and as it is an intrinsically weaker promoter than the CMV will give lower expression of the very potent cMYC transgene. This construct will be used as a basis for a DOD Idea Award application in the 2004 funding round.
- A tetracycline inducible 4th generation of LZRS construct (In collaboration with Dr. Xavier Stien) is also nearing construction.

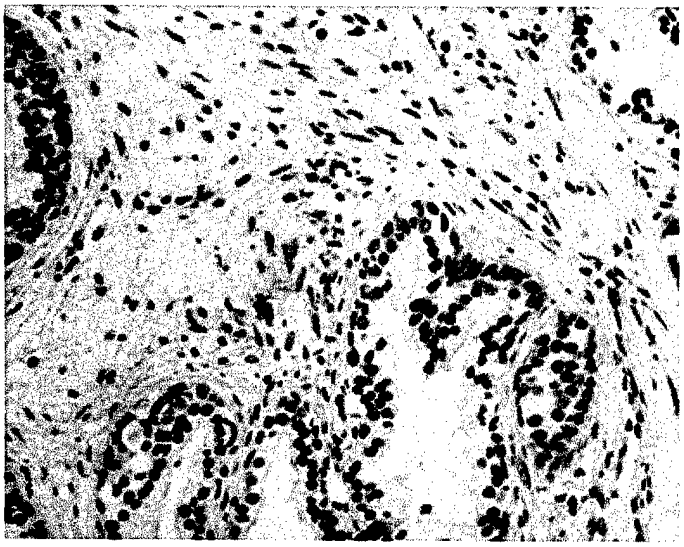
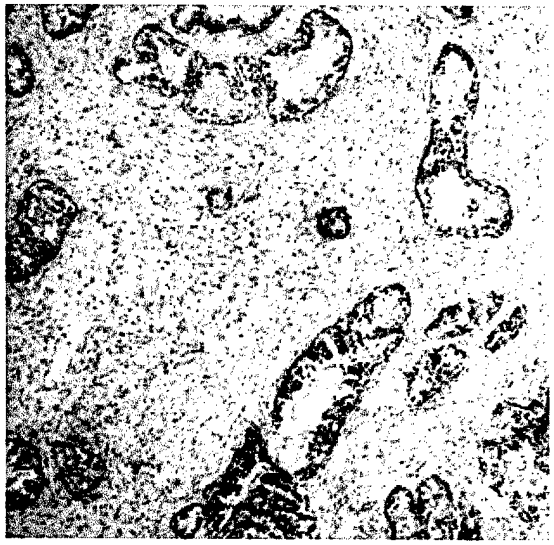
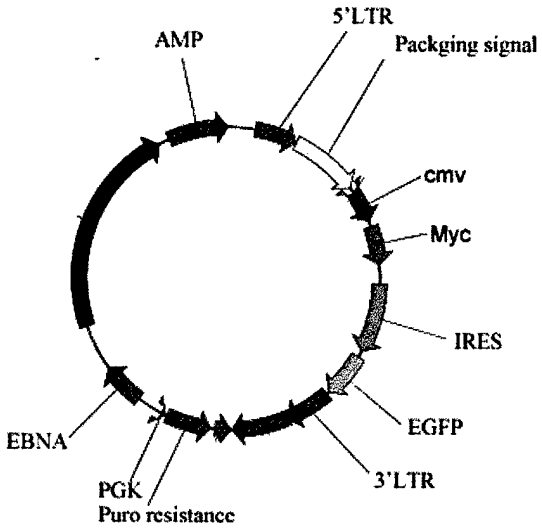


Fig. 1
Immunohistochemical analysis of ERβ in peripheral zone of human prostate



Immunohistochemical analysis of Nkx3.1 in apical zone of human prostate

Fig. 2
Schematic of LZRS retroviral construct carrying cMyc under the CMV promoter. The virus consists of only the RNA contained between the 5' and 3' LTR. Ordinarily the infected cells are FACS sorted to give a pure infected population but in the case of cMyc-infected cells this proved to be unnecessary. Upon infection the cells appear to enter the cell cycle and divide. The virus integrates when the cells divide therefore cMyc enhances the mechanism of integration resulting in very high levels of infection approaching 80% at the growing edge. Due to the rapid cell division the infected cells overwhelm the uninfected cells and a pure population is obtained in 3 weeks. The resultant population is not clonal and is quite diverse with respect to the expression levels of EGFP and subsequently cMyc expression.



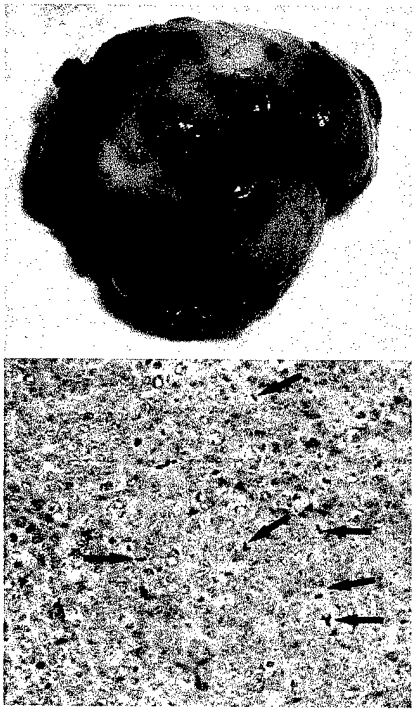


Fig. 3
huPrE/cMyc cells recombined with rUGM grafted under the renal capsule of a SCID mouse host. The host was sacrificed at 27 days due to size of graft, which exceeded 30mmdia. The graft displayed large areas of necrotic tissue in its interior with pockets of living tissue surrounding the blood vessels, a pattern reminiscent of all fast growing tumors that outgrow their ability to attract sufficient angiogenesis. Control grafts consisting of uninfected huPrE from the same patient were extremely small ≤2mm and had yet to form prostatic ducts (huPrE requires a minimum of 10 weeks to form well organized prostate ducts)
H&E of huPrE/ Myc +rUGM TR. The presence of many mitotic figures is readily apparent. Many abnormal mitotic figures are evident. This graft resembles a poorly differentiated adenocarcinoma. There was no evidence of invasive infiltration of this graft into the mouse kidney, however studies are ongoing.

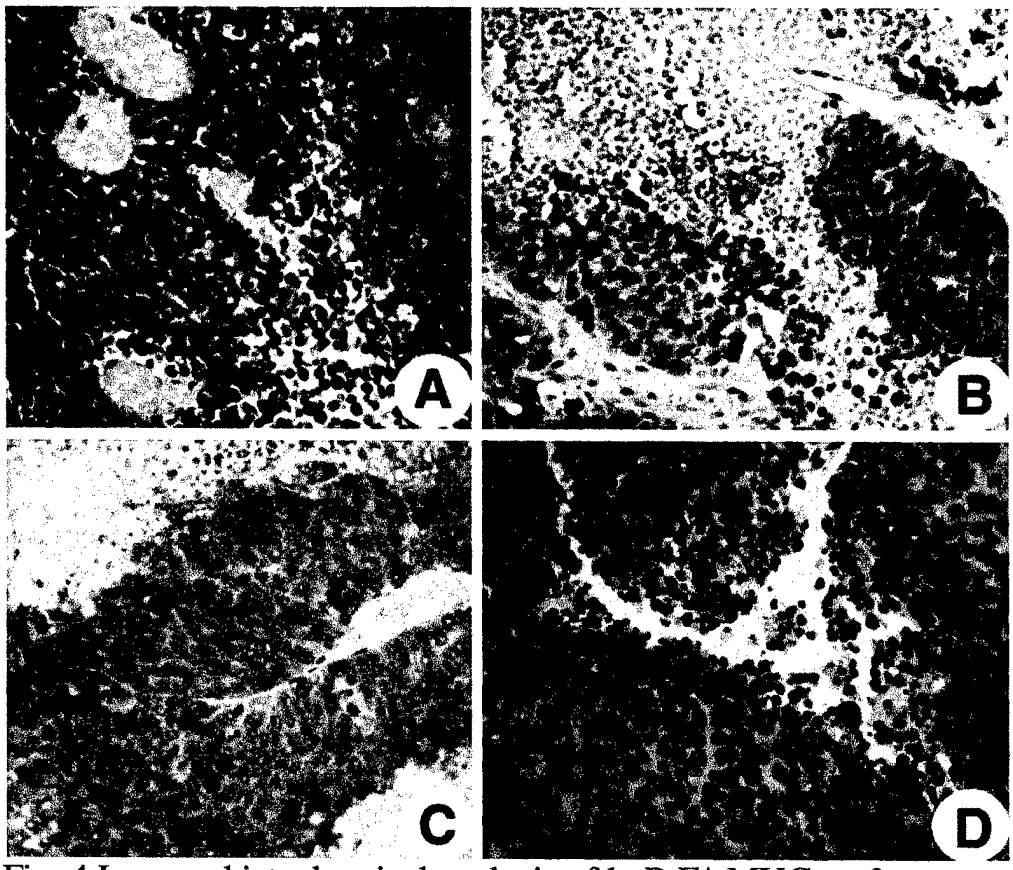
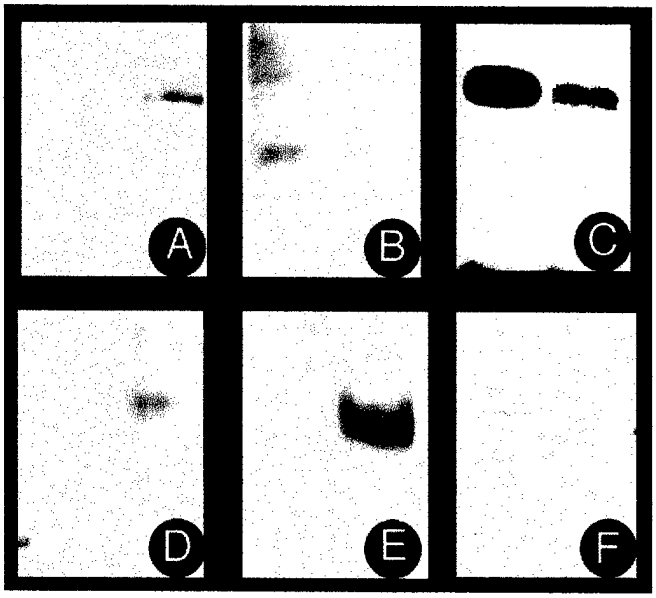


Fig. 4 Immunohistochemical analysis of huPrE/cMYC graft
(A) PSA (B) broad spectrum cytokeratin (C) EGFP (D) Ki67

Fig. 5
Western blot analysis of huPrE, (PZ cells in culture)
left lane. Right lane huPrE/c-myc, tumor tissue grown in SCID mouse. (A) EGFP; (B) E-Cad; (C) AR; (D) c-Myb; (E) c-Myc; (F) PTEN. All lanes contain 100 μg protein except PTEN, which contains 250 μg protein.

EGFP expression only in LZRS-Myc-EGFP

Expression of E-Cad⇓in tumor
Tumor expression of c-Myb ⇑⇑
Tumor expression of c-Myc ⇑⇑
PTEN expression of ⇓in tumor
Tumor is AR+



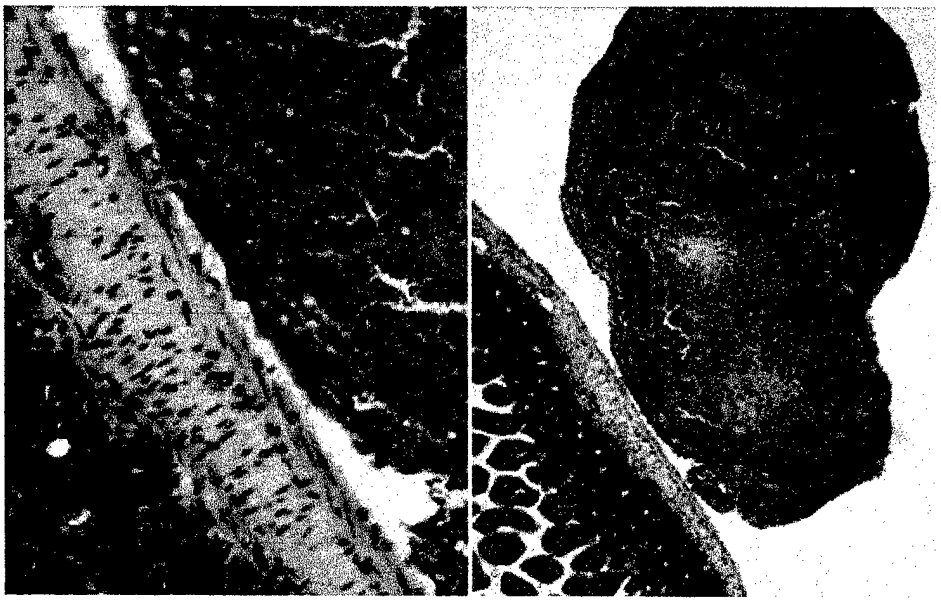


Fig. 6
huPrE/myc cells metastasized from the renal capsule and successfully seeded the abdominal cavity. Over 10 large nodules ≥2mm were observed in the vicinity of the intestine and liver. The histology of the nodules was consistent with that observed in the primary huPrE/myc tumor. These nodules were non invasive and their proximity to the organs appears to be primarily as a means to establish a blood supply. Hoechst 33258 staining (not shown) confirms that these nodules are not of mouse origin. EGFP IHC establishes the human origin of these nodules
Tumor nodules are PSA and ERβ positive

Training

I have attended and participated in the 2003 SBUR/ESUR Fall Meeting /5th World Congress and the 2001 SBUR/ESUR Fall Meeting /4th World Congress as well as the 2002 SBUR fall meeting. I presented posters at all three meetings and was the recipient of a travel award at the 2002 and 2003 meetings.

In July 2002 I was accepted to the AACR Pathobiology of Cancer 'Cancer Camp' in Keystone Colorado. This was a laboratory-based workshop at which I presented a poster and attended several round table discussions, while receiving training in the histopathologic and gross characterization of human cancer.

I was also a participant at the Joint Host-Tumor Interactions Program of the Vanderbilt Ingram Comprehensive Cancer Center & the Department of Cancer Biology Retreat, Lake Barkley, Kentucky. In 2001 I gave an oral presentation and in 2002 & 2003 I presented posters.

In Aug. 2001 I attended an AUA Research conference on benign urogenital tract disease hosted in Houston.

Laboratory meeting discussions and journal club presentations are integral to the running of the department as is interaction with a branching morphogenesis journal club run by the Department of Nephrology. I attend and have presented within this forum.

Lunchtime and evening seminars and mini symposiums as well as the Vanderbilt DOD Prostate Cancer Center Retreat also contribute to an overall training strategy in place within the department.

Reportable outcomes
Papers

Williams, K., Ishii, K., Shappel, S., Hayward SW: c-Myc overexpression in human prostate initiates cancer *in vivo*. Manuscript in preparation

Talks

Oct. 7, 2003 MRC Human Reproductive Sciences Unit (Edinburgh), Methods for profiling Prostate Cancer.

November 16-17, 2001 Joint Host-Tumor Interactions Program & Department of Cancer Biology Retreat, Lake Barkley, Kentucky. Human prostate carcinogenesis initiated by Retroviral gene transfer.

Awards

Travel award from the SBUR to attend the SBUR/ESUR 2003 Fall Meeting and the 5th World Congress in Urologic Research London Sept. 24-27, 2001

Travel award from the SBUR to attend the SBUR Fall Meeting, Tucson, AZ USA Dec. 5-8 Dec, 2002

Poster Abstracts

Karin Williams., Ishii K., & Hayward SW. November 21-22, 2002 3rd.Joint Host-Tumor Interactions Program & department of Cancer Biology Retreat, Lake Barkley, Kentucky. characterization of human prostatic epithelial cells infected with a retrovirus carrying the c-myc proto-oncogene

Williams, K., Ishii K. and Hayward SW (2003) 10th Annual Cap Cure Scientific meeting c-Myc overexpression in human prostate initiates cancer *in vivo* Washington DC Oct. (rescheduled) to New York Nov. 8-10

Williams, K., Ishii K. and Hayward SW (2003) characterization of human prostatic epithelial cells from the transitional & peripheral zone infected with a retrovirus carrying the c-myc proto-oncogene. SBUR/ESUR 2003 Fall Meeting and the 5th World Congress in Urologic Research London Sept. 24-27, 2001 Travel award recipient

Karin Williams., Suzanne Fernandez, Kenichiro Ishii, Simon W Hayward. (2002) Retroviral gene transfer into human prostatic epithelium permits investigation of putative oncogenes in a tissue recombination model. SBUR Fall Meeting, Tucson, AZ USA Dec. 5-8 Dec, 2002

Karin Williams., Fernandez S, Ishii K, Love H, Hayward S. November 15-16, 2002 2nd.Joint Host-Tumor Interactions Program & department of Cancer Biology Retreat, Lake Barkley, Kentucky. Retroviral gene transfer: a tool for the modern molecular biologist

Karin Williams., Chris Lau, Simon W Hayward.(2002) Human Prostate Cancer in a tissue Recombination model. Pathobiology of Cancer Keystone CO,USA July 14-21

Williams, K., Lau C., Hayward, SW (2001) Human Prostatic Carcinogenesis Initiated by Retroviral Gene Transfer SBUR/ESUR 2001 Fall Meeting and the 4th World Congress in Urologic Research Tucson, AZ USA Nov. 29-Dec 2, 2001

Book Chapters

Williams, K. and Hayward, S.W. Stem cells in glandular organs. In: Stem Cell Handbook (Ed Sell, S.) Humana Press, Totowa, N.J. 2003 chapter 28 307-315

Reagents Generated

Generation of several c-Myc overexpressing cell lines which are immortal at least one of which is AR +ve and produces PSA.